AD_____

Award Number: W81XWH-12-1-0391

TITLE: Meta-Analytical Online Repository of Gene Expression Profiles of MDS Stem Cells

PRINCIPAL INVESTIGATOR: Dr. Amit Verma, M.D.

CONTRACTING ORGANIZATION: Albert Einstein College of Medicine
Bronx, NY 10461-1602

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2013	Annual	30September2012—29September2013

4. TITLE AND SUBTITLE
Meta-Analytical Online Repository of Gene Expression Profiles of MDS Stem Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0391

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Amit Verma

E-Mail: amit.verma@einstein.yu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Albert Einstein College of Medicine
Cancer Center

Bronx, NY 10461-1602

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We propose to create an online searchable repository of gene expression profiles of CD34+ stem cells from patients with myelodysplastic syndromes (MDS) and healthy controls. We have devised a novel meta-analytical approach to integrate and normalize gene expression studies generated in different labs on different platforms and have shown it to be feasible and biologically valid in numerous publications. We have now successfully integrated data from 183 MDS samples in the database. This dataset has been used to screen for expression of all TGF-β related genes and has led to the discovery that negative regulator, SMAD7, was significantly underexpressed in MDS stem cells. This discovery elucidated the mechanism of activation of TGF-β signaling in MDS, even in the absence of high extracellular levels of TGF-β. After demonstrating the utility of this database in this study, we now propose to add information about MDS subtypes, blood counts, IPSS scores, patient demographics to this database. Investigators will be allowed to access this database online. This resource will continually be updated with newer data on an ongoing basis.

15. SUBJECT TERMS
MDS, Stem Cells, Gene Expression

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction:

MDS is a heterogeneous group of diseases characterized by bone marrow failure that leads to cytopenias. Newer therapeutic developments are impeded by limited insights into disease pathophysiology. Lack of cell lines and valid mouse models underscore the importance of research based on primary patient samples. These samples are hard to obtain in sufficient numbers. Our proposal will create a gene expression database that will include large numbers of all MDS subtypes. Moreover, this database will be generated by compiling gene expression profiles from CD34+ purified stem cells, thus ensuring that these profiles are not diluted due to heterogeneity of whole bone marrow samples. In addition to allowing researchers to study the expression patterns of selected genes, this database will also allow researchers to identify subtypes of MDS patients that will potentially benefit from novel therapies that target specific genes or genetic pathways.

Body:

We had previously shown that meta-analysis of microarray studies demonstrates feasibility of inter-platform data integration and reveals novel hematopoietic stem cell signatures. We tested the feasibility of conducting a meta-analysis of GE studies by using publically available data from studies that used normal bone marrow-derived hematopoietic progenitors. Data was integrated using both RefSeq and UniGene identifiers and normalized. We observed that in spite of variability introduced by experimental conditions and different microarray platforms, our meta-analytical approach can distinguish biologically distinct normal tissues by clustering them based on their cell of origin(1).

After demonstrating the feasibility of our meta-analytical approach, we wanted to construct a database of MDS stem cells and normal controls. We have now integrated data from 183 MDS CD34+ samples and 17 healthy controls. The data was integrated using unigene IDs, normalized and shown to be valid for further analysis (2). We have subsequently used this database in 4 studies that have led to important insights into the pathophysiology of MDS (2-5). In the first study, we showed that the SMAD2 protein is overexpressed in MDS. This protein is an effector of the TGF-β signaling pathway and is activated by the TGF-β receptor I kinase. In the next two studies, we wanted to determine the reasons for activation of TGF- β signaling pathway in MDS stem cells. Therefore, we used the MDS gene expression database to screen for expression of all TGF- β related genes and discovered that the negative regulator, SMAD7, was significantly underexpressed in MDS stem cells (3, 5). SMAD7 protein is an endogenous inhibitor of the TGF-β receptor I kinase. Functional studies revealed that reduction in SMAD7 (observed in the meta-analysis) leads to overactivation of the TGF-β receptor kinase even in the absence of high extracellular levels of the cytokine. These studies thus established SMAD7 reduction as a key intracellular event that leads to myelosuppressive TGF-β signaling and ineffective hematopoiesis in MDS (3, 5).

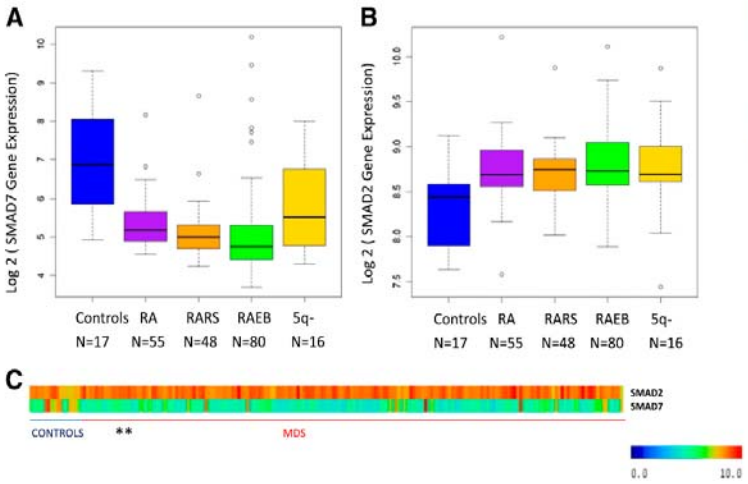

Figure 1. *SMAD7* expression is significantly decreased in MDS CD34+ cells. (A) SMAD7 expression in 183 samples of MDS CD34+ cells and 17 healthy controls reveals reduction in all subsets of MDS. (false discovery rate < 0.1, Benjamin Hochberg correction multiple testing). (B) *SMAD2*, the effector SMAD protein for TGF-β signaling, was found to be increased in the same samples. (C) Heatmaps showing expression values for both genes. (The 5q- patients were a subset of the RA patient cohort.)

In another study, we determined that DOCK4, a GTPase exchange factor is underexpressed in MDS (Fig 2). This study also utilized the database we created to show DOCK4 underexpression in a large number of MDS samples (Fig 2)(Shown at Fig5 from ref 4).

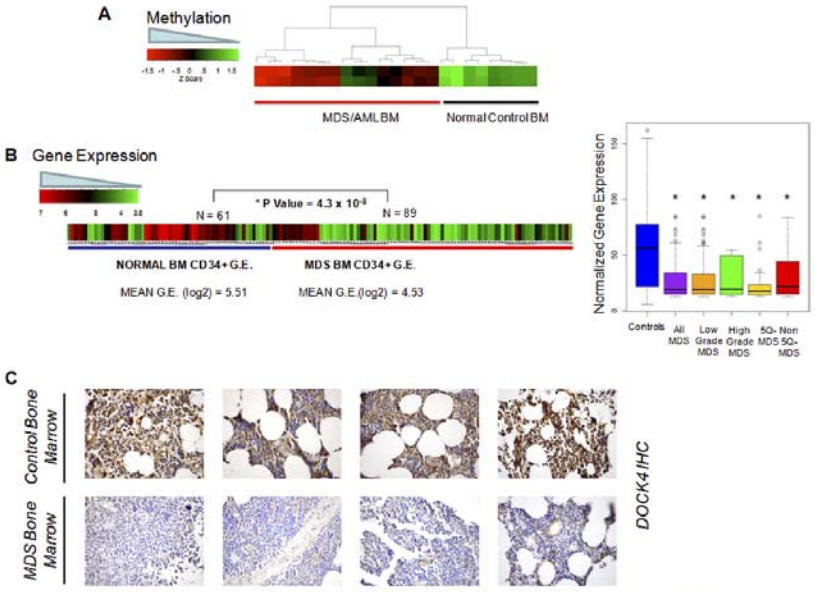

FIGURE 5. **Validation in independent cohorts demonstrate reduction in DOCK4 in marrow samples from MDS/AML.** Methylation values obtained from the HELP assay performed on marrow (*BM*) samples in an independent cohort of patients (38) show hypermethylation of the promoter in MDS/AML samples (*A*). Gene expression values from various studies on MDS and normal bone marrow-derived CD34$^+$ cells were obtained and normalized. Mean expression of *DOCK4* was significantly reduced in 89 MDS cases when compared with 61 controls (two-tailed *t* test) (*B, left panel*); box plots of MDS subtypes show significantly reduced levels of DOCK4 in all subtypes of MDS (*B, right panel*). Bone marrow biopsy samples were stained with DOCK4 antibody and show decreased expression in four representative cases of MDS when compared with controls (*C*).

After demonstrating the utility of this database in this study, we now propose to add information about MDS subtypes, blood counts, IPSS scores, patient demographics to this database. Investigators will be allowed to access this database online. This resource will continually be updated with newer data on an ongoing basis.

Key Research Accomplishments:

We have shown the feasibility of constructing a meta-analytical database of MDS stem cell samples and controls, and have shown that this database can be used in basic as well as translation studies in MDS.

Reportable Outcomes:

Manuscript:

Bhagat T, Zhou L, Sokol L, Caceres G, Gundabolu K, Gordon S, Mantzaris I, Gligich O, Yu Y, Bhattacharyya S, Jing X, Polineni R, Tamari R, Bhatia K, Pellagatti A , Boultwood J, Kambhampati S, Steidl U, Stein C, Ju W, Liu G, Kenny P, List A, Bitzer M, **Verma A.** miR-21 mediates hematopoietic suppression in MDS by activating TGF- β signaling ***Blood*** 2013, Apr 11;121(15):2875-81. PMID: 23390194

Conclusion:

We have shown the feasibility of constructing a meta-analytical database of MDS stem cell samples and controls, and have shown that this database can be used in basic as well as translation studies in MDS. We will now incorporate clinical information to this database in the new phase of the funding period.

References:

1. Sohal, D., Yeatts, A., Ye, K., Pellagatti, A., Zhou, L., Pahanish, P., Mo, Y., Bhagat, T., Mariadason, J., Boultwood, J., et al. 2008. Meta-analysis of microarray studies reveals a novel hematopoietic progenitor cell signature and demonstrates feasibility of inter-platform data integration. *PLoS ONE* 3:e2965.
2. Zhou, L., Nguyen, A.N., Sohal, D., Ying Ma, J., Pahanish, P., Gundabolu, K., Hayman, J., Chubak, A., Mo, Y., Bhagat, T.D., et al. 2008. Inhibition of the TGF-beta receptor I kinase promotes hematopoiesis in MDS. *Blood* 112:3434-3443.
3. Bhagat, T.D., Zhou, L., Sokol, L., Kessel, R., Caceres, G., Gundabolu, K., Tamari, R., Gordon, S., Mantzaris, I., Jodlowski, T., et al. 2013. miR-21 mediates hematopoietic suppression in MDS by activating TGF-beta signaling. *Blood*.
4. Zhou, L., Opalinska, J., Sohal, D., Yu, Y., Mo, Y., Bhagat, T., Abdel-Wahab, O., Fazzari, M., Figueroa, M., Alencar, C., et al. 2011. Aberrant epigenetic and genetic marks are seen in myelodysplastic leukocytes and reveal Dock4 as a candidate pathogenic gene on chromosome 7q. *J Biol Chem* 286:25211-25223.
5. Zhou, L., McMahon, C., Bhagat, T., Alencar, C., Yu, Y., Fazzari, M., Sohal, D., Heuck, C., Gundabolu, K., Ng, C., et al. 2011. Reduced SMAD7 leads to overactivation of TGF-beta signaling in MDS that can be reversed by a specific inhibitor of TGF-beta receptor I kinase. *Cancer Res* 71:955-963.

Appendices:

None